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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PS 1606 for a patent by THE UNIVERSITY OF QUEENSLAND as filed on 08 April 2002.



WITNESS my hand this
Fifteenth day of April 2003

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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PROVISIONAL SPECIFICATION

Applicants:

THE UNIVERSITY OF QUEENSLAND

Invention Title:

THERAPEUTIC METHOD

The invention is described in the following statement:

THERAPEUTIC METHOD

FIELD OF THE INVENTION

This invention relates to the use of a C5a receptor antagonist, for example a cyclic peptide antagonist of the C5a receptor, in the prevention and/or treatment of fibrosis, such as the treatment of fibrosis associated with myocardial infarction or diabetes.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 60% of known cellular receptor types. They mediate signal transduction across the cell membrane for a very wide range of endogenous ligands and consequently participate in a diverse array of physiological and pathophysiological processes, including, but not limited to, those associated with cardiovascular, central and peripheral nervous system reproductive, metabolic, digestive, immunoinflammatory, and growth disorders, as well as other cell regulatory and proliferative disorders. Agents which selectively modulate functions of G protein-coupled receptors have the potential for therapeutic applications. These receptors are becoming

increasingly recognised as important drug targets, due to their crucial roles in signal transduction (G protein-coupled receptors, IBC Biomedical Library Series, 1996)

One of the most intensively studied G protein-coupled receptors is the receptor for C5a. C5a is one of the most potent chemotactic agents known, recruiting neutrophils and macrophages to sites of injury, altering their morphology; inducing degranulation; increasing calcium mobilisation, vascular permeability (oedema) and neutrophil adhesiveness; contracting smooth muscle; stimulating the release of inflammatory mediators, including histamine, TNF- α , IL-1, IL-6, IL-8, prostaglandins, and leukotrienes, and of lysosomal enzymes; promoting the formation of oxygen radicals; and enhancing antibody production (Gerard and Gerard, 1994).

Overexpression or underregulation of C5a is implicated in the pathogenesis of immune system-mediated inflammatory conditions, such as rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, lung injury and extracorporeal post-dialysis syndrome, and in a variety of other conditions (Whaley 1987; Sim 1993).

Agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these reasons, molecules which prevent C5a from binding to its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Such compounds also provide valuable new insights into the mechanisms of complement-mediated immunity.

Fibrosis, the ingrowth of fibroblasts and the production of extracellular matrix to form abnormal scarring, can result from many causes, including trauma, surgical interventions, infections and pathological

conditions. Fibrosis is a sequel of conditions such as chronic inflammation, including inflammation arising from diabetes and hypertension. It can occur in variety of tissues, including but not limited to the lung, kidney, liver and heart. Fibrosis contributes to the loss of function experienced in such conditions, through the formation of abnormal quantities of extracellular matrix which change the physical properties of the scarred tissue. Diabetes- or hypertension-induced fibrosis of the heart, for instance, produces stiffening of the ventricle walls that contributes to decreased cardiac output.

It is estimated that 45% percent of deaths in the USA are attributable to disorders exhibiting proliferative fibrosis. Although fibrosis is debilitating and may be life-threatening, and the number of individuals who may benefit from an effective antifibrotic therapy is large, currently there are no effective treatments available.

Fibrosis is a dynamic process, and is considered to be potentially reversible. The extracellular matrix laid down during fibrosis may be resorbed after the withdrawal of the fibrotic stimuli. In many cases, however, the presence of fibrosis is only identified after loss of function has already taken place, for instance where decreased cardiac output is a sign of otherwise undetected cardiac fibrosis. Consequently, while it is desirable in certain circumstances to be able to prevent fibrosis from occurring, it is also desirable to be able to reverse existing fibrosis once it is detected.

The effects of drug-induced and hypertension-induced pulmonary and renal fibrosis in animal models can be prevented or partially reversed by compounds which act by suppressing inflammatory events and down-regulating lung pro-collagen I over-expression.

We have shown that the administration of pirfenidone or spironolactone can prevent and partially reverse cardiac fibrosis and the increase in cardiac

stiffness which occurs in streptozotocin-induced diabetes in rats. It is thought that pirfenidone acts by inhibiting increased TGF- β mRNA expression, allowing an increase in expression of metalloproteases which degrade the collagen I laid down during fibrosis. The mode of action of spironolactone is at present unknown. Spironolactone is a steroid analogue which is primarily used as a diuretic; pirfenidone is being investigated as an anti-fibrotic in a number of indications.

It would be highly desirable to identify other therapeutically or prophylactically active agents for use in the treatment or prevention of fibrosis.

The overexpression or underregulation of a G-protein-coupled receptor, the C5a receptor, has been implicated in immune-system mediated events such as inflammation. Agents which influence C5a receptor activity, such as C5a receptor antagonists, have the potential to mediate inflammatory events, and may provide a means of therapeutic or prophylactic intervention, but have not previously been suggested as potential agents in the treatment or prevention of fibrosis.

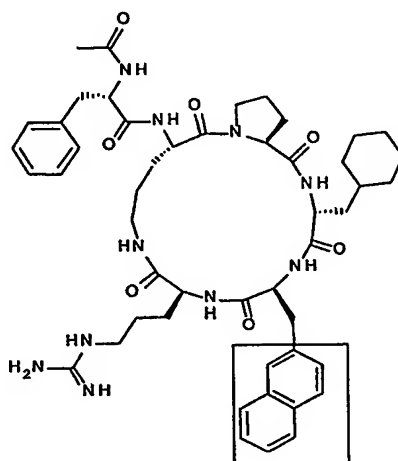
We have now found that a cyclic peptide with C5a receptor antagonist has the ability to ameliorate cardiac fibrosis in an animal model of this condition.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of prevention, treatment or alleviation of a fibrotic condition, comprising the step of administering an effective amount of a C5a receptor antagonist to a subject in need of such treatment.

The use of any compound having activity as a C5a receptor antagonist is contemplated, including but not limited to those disclosed in our earlier applications International patent application No. PCT/AU98/00490 or Australian provisional patent application No. PR8334 or in

patent application No. PCT/US/11187 in the name of Neurogen Corporation, No. PCT/JP01/06902 by Welfide Corporation, or antibody antagonists such as those disclosed in PCT/US00/24219 or US 6355245. The entire disclosures of all of these specifications are incorporated herein by this cross-reference. More preferably the C5a receptor antagonist is a peptide or a peptidomimetic compound, and more preferably is a cyclic peptide or a cyclic peptidomimetic compound. Most preferably the compound is the compound designated PMX53, disclosed in PCT/AU98/00490, which has the formula



In a second aspect, the invention provides the use of a C5a receptor antagonist for the manufacture of a medicament for use in the treatment of a fibrotic condition.

For the purposes of this specification, the term "C5a receptor antagonist" includes any compound which can reduce or inhibit effects mediated by the interaction between C5a and C5a receptor. Thus the term includes polyclonal or monoclonal antibodies, peptides, peptidomimetics, and non-peptide compounds.

Methods and pharmaceutical carriers for

preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

The compounds may be administered at any suitable dose and by any suitable route. Oral, transdermal or intranasal administration is preferred, because of the greater convenience and acceptability of these routes. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of health of the individual treatment. This will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

The carrier or diluent, and other excipients, will depend on the route of administration, and again the person skilled in the art will readily be able to determine the most suitable formulation for each particular case.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

For the purposes of this specification, the term "fibrotic condition" is to be taken to mean any fibrotic disorder, such as multiple sclerosis, retinal disorders including proliferative vitroretinopathy and macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of major organs such as the kidney, liver, heart or lungs.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

While it is particularly contemplated that the subject for treatment by the method of the invention is

human, the treatment is also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as felids, canids, bovids, and ungulates.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a. Daily water intake for control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. Arrow indicates initiation of L-NAME treatment.

Figure 1b. Daily L-NAME intake for L-NAME and L-NAME+C5a receptor antagonist treated rats.

Figure 2. Body weight of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. Arrow indicates initiation of L-NAME treatment.

Figure 3. Systolic blood pressure measurements of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist at day 32. Values expressed as mean \pm SEM. * $p < 0.05$ compared to control. ** $p < 0.05$ compared to L-NAME.

Figure 4. Left ventricular wet weight of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist. Values expressed as mean \pm SEM. * $p < 0.05$ compared to control.

Figure 5a. Interstitial collagen deposition in the left ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. * $p < 0.05$ compared to control; ** $p < 0.05$ compared to L-NAME.

Figure 5b. Perivascular collagen deposition in the left ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. * $p < 0.05$ compared to control; ** $p < 0.05$ compared to L-NAME.

Figure 6a. Interstitial collagen deposition in the right

ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 6b. Perivascular collagen deposition in the right ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 7a. Tubulointerstitial collagen deposition in the kidneys of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 7b. Glomerular collagen deposition in the kidneys of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 8a. Inflammatory cell count in the left ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 8b. Inflammatory cell count in the right ventricle of control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM. *p <0.05 compared to control; ** p <0.05 compared to L-NAME.

Figure 9. Echocardiographic data for control, control+C5a antagonist, L-NAME and L-NAME+C5a receptor antagonist treated rats. *p<0.05 compared to control; **p<0.05 compared to L-NAME.

9a. Left ventricular wall thickness in diastole.

9b. Left ventricular internal diameter in diastole.

9c. E/A flow ratio.

9d. Diastolic volume

9e. Cardiac output

Figure 10. Diastolic stiffness constants for control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values expressed as mean \pm SEM. *p < 0.05 compared to control; **p < 0.05 compared to L-NAME.

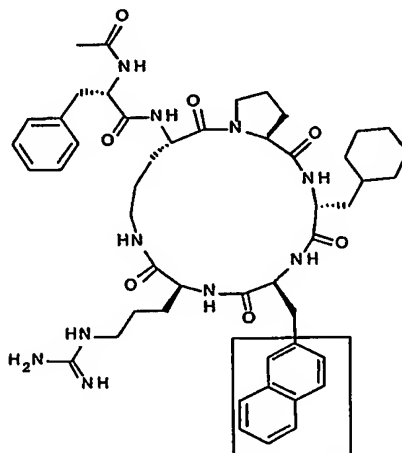
Figure 11. Developed pressure for control, control+C5a antagonist, L-NAME and L-NAME+C5a antagonist treated rats. Values are expressed as mean \pm SEM.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples and figures.

Example 1 Effect of a C5a receptor antagonist on L-NAME-induced cardiac fibrosis

Male Wistar rats (8 weeks old) were obtained from the Central Animal Breeding House of The University of Queensland. The rats were administered a C5a receptor antagonist designated PMX53, which has the formula:



This agent was administered at a dosage of 1mg/kg/day orally for 4 days before rats were additionally treated with L-nitroarginine methyl ester (L-NAME) for 4 weeks, ie a total duration of treatment of antagonist of 32 days. L-NAME administration produces hypertension and cardiac remodelling by inhibition of the production of nitric oxide (NO).

L-NAME (nitro-L-arginine methyl ester) was administered at a concentration of 400mg/l in the drinking water for 4 weeks to give a mean daily intake of 18.7 ± 0.4 mg L-NAME (41.4 ± 0.8 mg/kg mean body weight). Body weight and food and water intakes were measured daily.

Neither L-NAME nor C5a receptor antagonist treatment altered water intake or growth rate, as shown in Figures 1 and 2.

Systolic blood pressure was measured in selected unanaesthetised rats, using a tail-cuff method. As illustrated in Figure 3, systolic blood pressure increased from 118 ± 3 mmHg to 160 ± 2 mmHg in L-NAME-treated rats without significantly altering heart rate or increasing left ventricular weight, as determined by echocardiograph or post-mortem examination, when compared to control rats. These results are shown in Figure 4.

Similarly, right ventricular and other major organ weights were not significantly altered with L-NAME treatment.

C5a receptor antagonist treatment of L-NAME rats significantly increased systolic blood pressure by 16mmHg to 176 ± 3 mmHg, resulting in an increased left ventricular wet weight. Additionally, C5a receptor antagonist treatment of control rats induced a non-significant increase in blood pressure. These results are summarised in Figures 3 and 4. C5a receptor antagonist treatment of both control and L-NAME rats did not significantly alter wet weights of the remaining major organs.

After 4 weeks of L-NAME treatment, heart function

was determined *in vivo* by echocardiography and *in vitro* using the isolated Langendorff heart preparation described below. Collagen deposition was measured by image analysis using laser confocal microscopy of picrosirius red-stained cardiac slices as described below.

Rats were euthanased with pentobarbitone (100 mg/kg ip). Blood was taken from the abdominal vena cava, centrifuged and the plasma frozen. Plasma glucose was measured by Precision Plus Blood Glucose Electrodes (Medisense, Abbott Laboratories); plasma Na^+ and K^+ were measured by flame photometry. Plasma angiotensinogen and renin concentrations were measured as previously described (Marchant et al, 1993); aldosterone was measured using a commercial radioimmunoassay kit.

a) Collagen distribution

Collagen distribution was determined by image analysis of sections of heart and kidney stained with picrosirius red (0.1% Sirius Red F3BA in picric acid) which selectively stains fibrillar collagen. Slides were left in 0.2% phosphomolybdic acid for 5 minutes, washed, left in picrosirius red for 90 minutes, then in 1 mM HCl for 2 minutes and 70% ethanol for 45 seconds. The stained sections were analyzed with an Image Pro plus analysis program using an Olympus BH2 microscope, with results expressed as a percentage of red area in each screen. At least 4 areas were examined in each heart.

Image analysis showed an increase of 108% in interstitial collagen and an 87% increase in perivascular collagen in the left ventricle of L-NAME treated rats when compared to controls. Similarly, a significant increase in collagen levels was observed in the right ventricle, where a 175% increase in interstitial and a 37% increase in perivascular collagen content occurred. L-NAME treatment also significantly increased the collagen content by 55% in the tubulointerstitial areas of the kidneys with a smaller

increase in glomerular spaces. These results are presented in Figures 5, 6 and 7.

C5a receptor antagonist treatment attenuated the increased collagen deposition. In C5a antagonist treated rats, L-NAME treatment produced 23% and 43% of the increase observed in rats treated with L-NAME only when comparing the left ventricular interstitial and perivascular areas respectively. Similar results were observed in the right ventricle, where C5a receptor antagonist treatment of L-NAME restricted collagen deposition to 44 and 37% in the interstitial and perivascular areas respectively. In the kidneys, C5a antagonist administration to L-NAME rats restricted collagen deposition to 30% in the interstitium and normalized the increase in glomerular collagen concentrations observed in L-NAME treated rats. The results are presented in Figures 5, 6 and 7.

L-NAME treatment resulted in a large inflammatory cell infiltration in both the left and right ventricles. A 30-fold increase in inflammatory cell population was observed in the both left and right ventricular interstitial and perivascular areas following L-NAME treatment. C5a receptor antagonist treatment totally prevented inflammatory cell infiltration into left or right ventricles following L-NAME treatment. This result is summarised in Figure 9. No information is available on inflammatory cell type or kidney infiltration.

b) Fibronectin concentrations

Fibronectin concentrations were determined in hearts stored at -10°C in phenylmethyl-sulphonyl fluoride (0.1M). Hearts were ground and centrifuged to separate soluble and insoluble fibronectin. A sandwich enzyme-linked immunosorbent assay (ELISA) protocol was followed using a rabbit antihuman fibronectin antibody in a carbonate buffer, and sheep antirabbit fibronectin conjugated to horse radish peroxidase. Colour formation

was measured at 495 nm after addition of o-phenylenediamine and hydrogen peroxide. Concentrations were read against appropriate standards.

c) Echocardiographic analysis

Cardiac function was estimated *in vivo* using echocardiography, using conventional methods.

Although L-NAME treatment did not significantly increase left ventricular weight, echocardiographic M-mode measurements showed that L-NAME treatment had triggered cardiac remodelling, increasing the left ventricular wall thickness and decreasing the left ventricular internal diameter in diastole. Further L-NAME treatment significantly increased the ratio of early (E) to atrial (A) mitral valve inflow rates (E/A ratio), and significantly decreased diastolic volume and cardiac output. Fractional shortening and ascending aortic flow rates were not significantly altered by L-NAME treatment. Thus, L-NAME treatment produces cardiac remodelling with minor changes in systolic function and an improved diastolic function.

C5a receptor antagonist treatment of control rats did not significantly alter any parameter measured by echocardiographic analysis. C5a receptor antagonist treatment of L-NAME rats normalised the increase in left ventricular wall thickness and decreased left ventricular internal dimensions. This treatment also significantly normalised the E/A ratio, diastolic volume and cardiac output. These results are presented in Figure 9.

d) Isolated Langendorff heart preparation

The Langendorff isolated heart preparation was used to determine the diastolic stiffness of the left ventricles *ex vivo*.

Rats were anaesthetised with sodium pentobarbitone (100mg/kg ip) and heparin (2000 IU) was administered via

the femoral vein. After allowing 2 minutes for the heparin to fully circulate, the heart was excised and placed in cooled (0°C) crystalloid perfusate (Krebs-Henseleit solution of the following composition in mM: NaCl 118, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.3, NaHCO₃ 25.0, glucose 11.0. The heart was then attached to the cannula (with the tip of the cannula positioned immediately above the coronary ostia of the aortic stump) and perfused in a non-recirculating Langendorff fashion at 100cm of hydrostatic pressure. The buffer temperature was maintained at 35°C. The hearts were punctured at the apex to facilitate thebesian drainage and paced at 250 bpm.

A balloon catheter was inserted in the left ventricle via the mitral orifice for measurement of left ventricular developed pressure. The catheter was connected via a three-way tap to a micrometer syringe and to a Statham P23 pressure transducer. The outer diameter of the catheter was similar to the mitral annulus to prevent ejection of the balloon during the systolic phase. After a 10 minute stabilisation period, steady-state left ventricular pressure was recorded from isovolumetrically beating hearts. Increments in balloon volume were applied to the heart until left ventricular end-diastolic pressure reached approximately 30mmHg.

To assess myocardial stiffness in isolated Langendorff hearts, stress (σ , dyne/cm²) and tangent elastic modulus (E, dyne/cm²) for the midwall at the equator of the left ventricle were calculated by assuming spherical geometry of the ventricle and considering the midwall equatorial region as representative of the remaining myocardium:

$$\sigma = \frac{VP}{W} \left(1 + \frac{4(V+W)}{[V^{1/3} + (V+W)^{1/3}]^3} \right)$$

$$E = 3 \left\{ \frac{VP}{W} - \sigma + \frac{\left[\frac{\sigma}{V} + \frac{(W\sigma - VP)}{W(V+W)} + \frac{\sigma \cdot dP}{P \cdot dV} \right] \times [V^{1/3} + (V+W)^{1/3}]}{[V^{-2/3} + (V+W)^{-2/3}]} \right\}$$

where V is chamber volume (ml), W is left ventricular wall volume (0.943 ml/g ventricular weight) and P is end diastolic pressure (dyne/cm²=7.5x10⁻⁴ mmHg). Myocardial diastolic stiffness is calculated as the diastolic stiffness constant (k, dimensionless), the slope of the linear relation between E and σ (Mirsky and Parmley, 1973). To assess contractile function, maximal +dP/dt was calculated at a diastolic pressure of 5 mmHg.

All results are given as mean \pm SEM of at least 6 experiments. The negative log EC50 of the increase in either force of contraction in mN or rate of contraction in beats/min was determined from the concentration giving half-maximal responses in individual concentration-response curves. Renal function results were corrected for kidney wet weight measured at the end of the experiment. These results were analysed by two-way analysis of variance followed by the Duncan test to determine differences between treatment groups and by paired or unpaired t-tests as appropriate; p<0.05 was considered significant.

At the end of the experiment, the atria and right ventricle were dissected away and the weight of the left ventricle plus septum was recorded.

L-NAME treatment markedly increased the diastolic stiffness constant of the ventricles when compared to controls. Developed pressure and contractility were not altered by L-NAME treatment. C5a receptor antagonist

treatment prevented the increased diastolic stiffness constant of L-NAME rats without altering contractility or developed pressure. These results are presented in Figures 10 and 11.

e) Isolated cardiac muscles and thoracic aortic rings

The heart was removed under anaesthesia. The right atria and papillary muscles from the left ventricle were removed and suspended in organ baths at a resting tension of 5-10 mN adjusted to give the maximal twitch response. Tissues were bathed in a modified Tyrodes solution (in mM): NaCl 136.9, KCl 5.4, MgCl₂ 1.05, CaCl₂ 1.8, NaHCO₃ 22.6, NaH₂PO₄ 0.42, glucose 5.5, ascorbic acid 0.28, sodium edetate 0.05, bubbled with 95% O₂/5%CO₂ and stimulated at 1Hz at 35°C as previously described (Brown et al, 1991a). Cumulative concentration-response curves were measured for noradrenaline and, following washout and re-equilibration, to calcium chloride. At the end of the experiment, papillary muscle dimensions were measured under the loading conditions of the experiment; all tissues were blotted and weighed.

Thoracic aortic rings (approximately 4 mm in length) were suspended with a resting tension of 10 mN (Brown et al, 1991b) and contracted twice with isotonic KCl (100 mM). The presence of endothelium was demonstrated by addition of acetylcholine (1×10^{-5} M). Cumulative contraction responses to noradrenaline were measured. Separate thoracic aortic rings were perfused with 10% neutral buffered formalin, embedded in wax and stained with haemotoxylin and eosin. Image analysis (Wild-Leitz MD30+ system) was used to calculate wall area of the thoracic aorta.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments

and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

Figure 1a

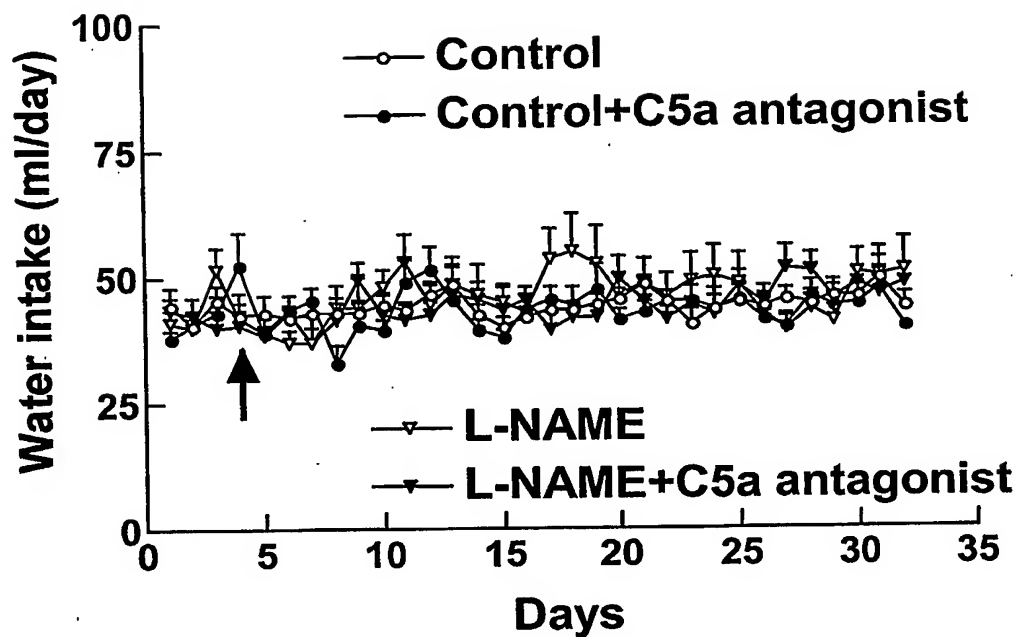


Figure 1b

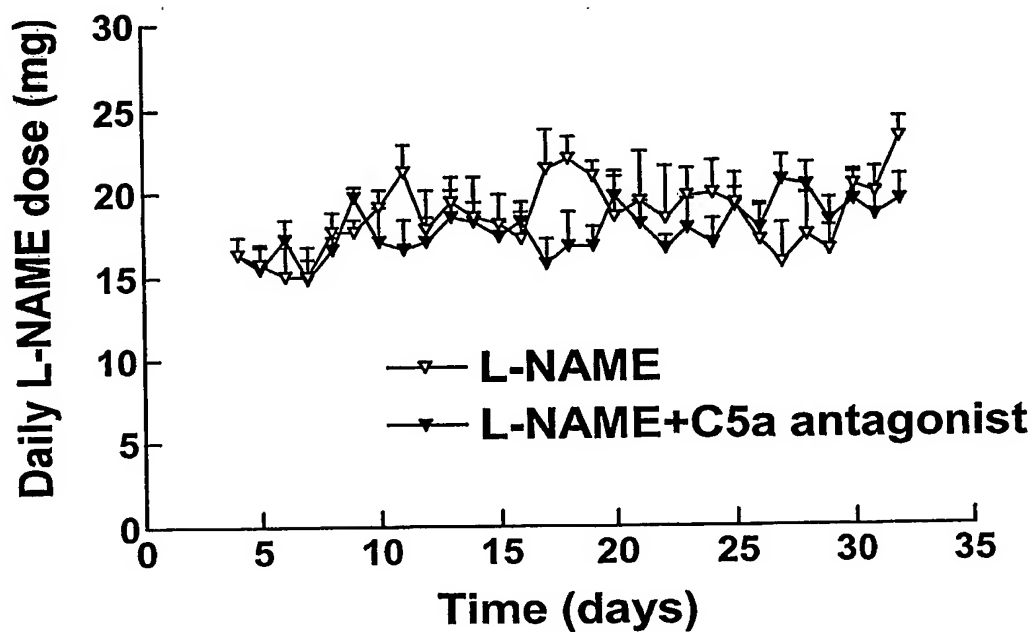


Figure 2

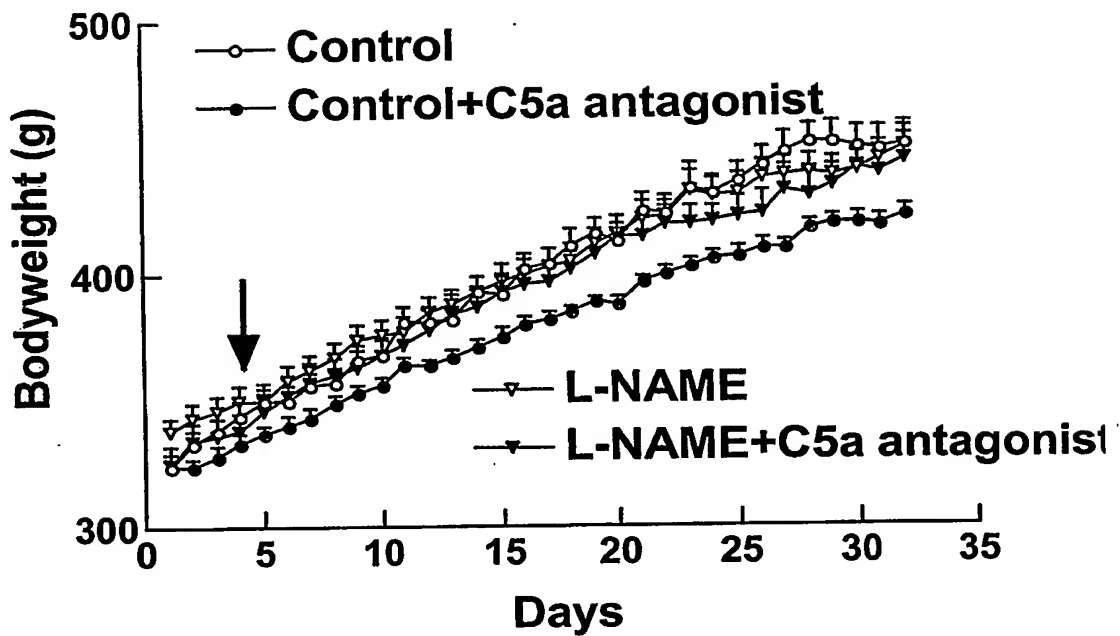


Figure 3

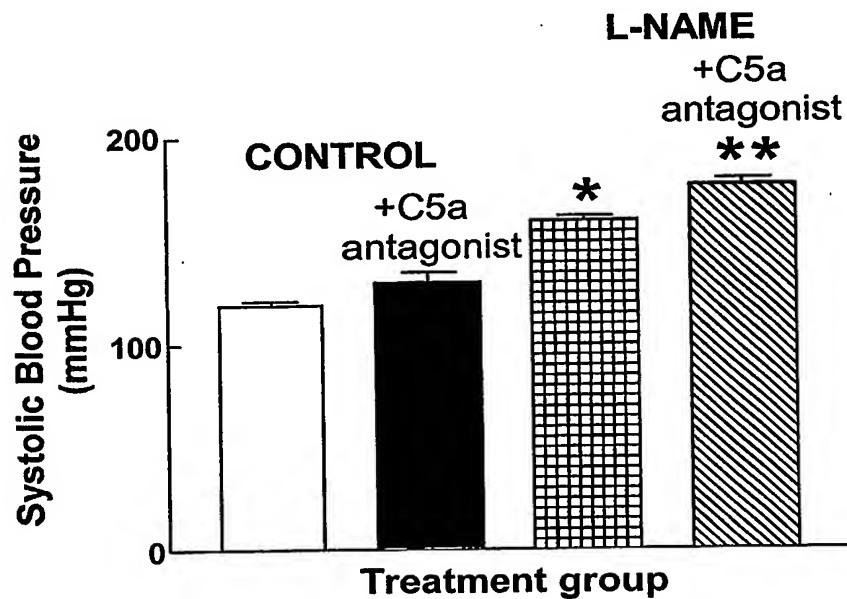


Figure 4

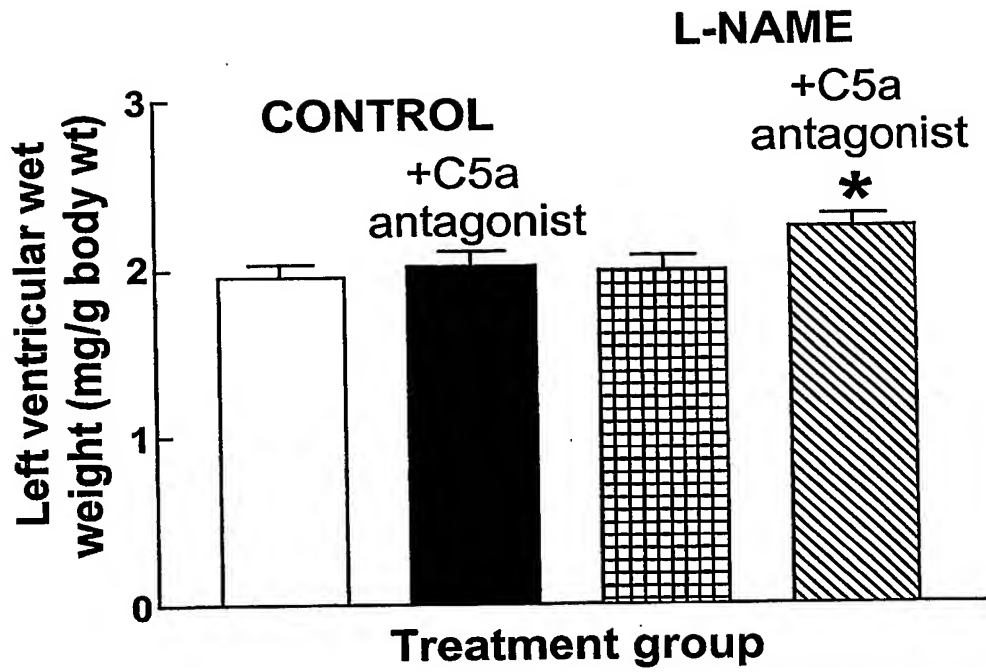


Figure 5a

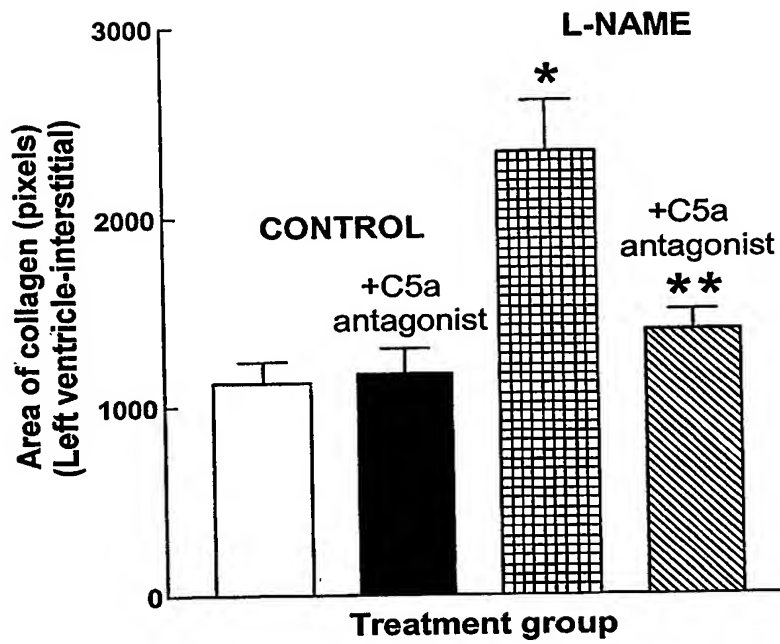


Figure 5b

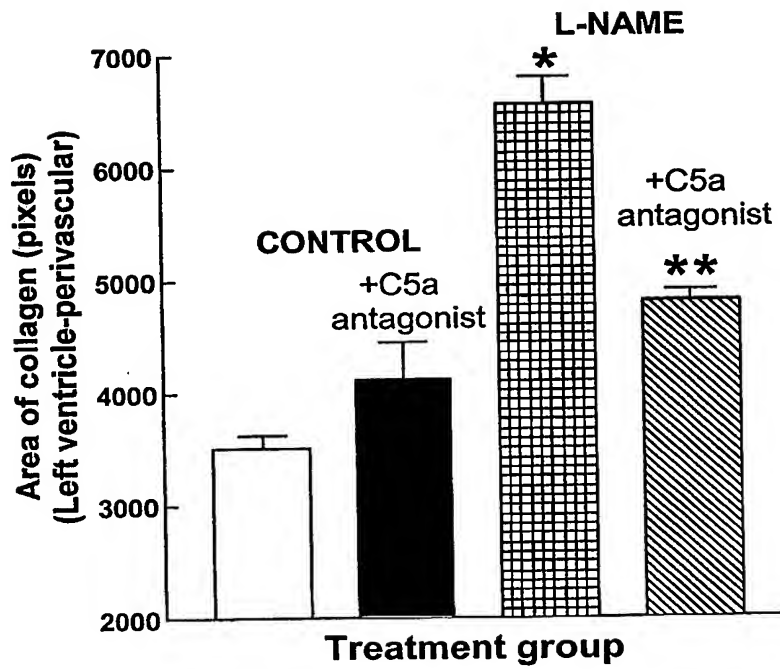


Figure 6a

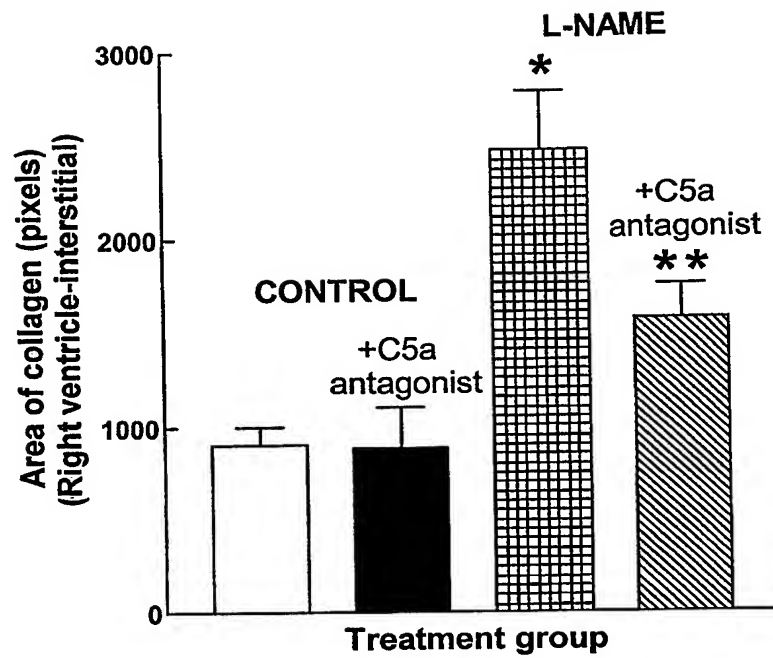


Figure 6b

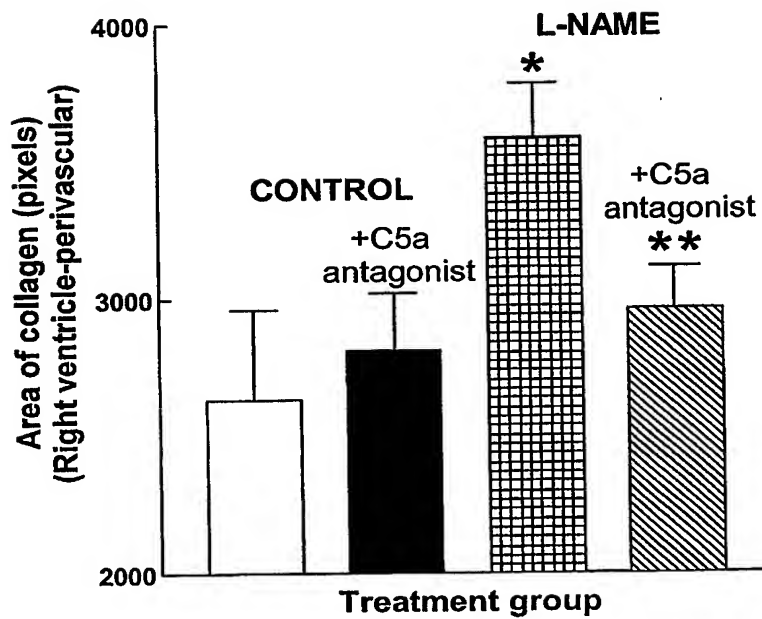


Figure 7a

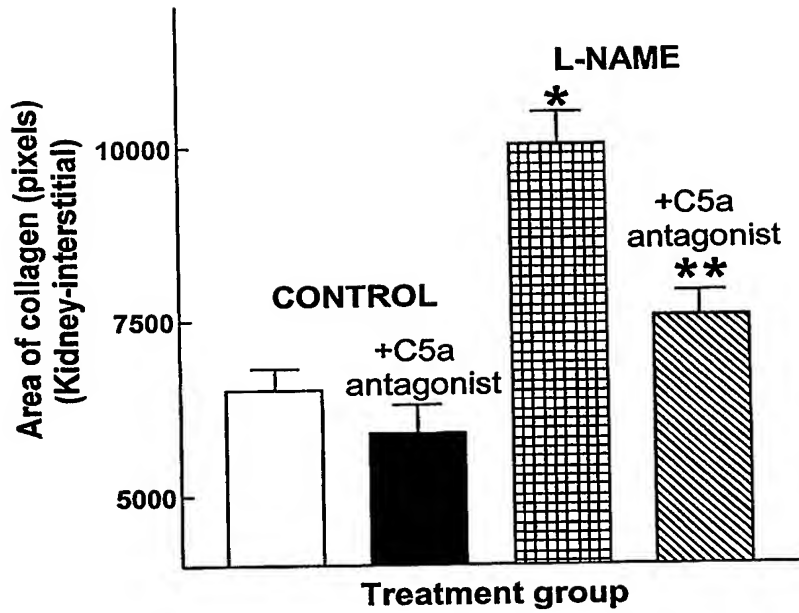


Figure 7b

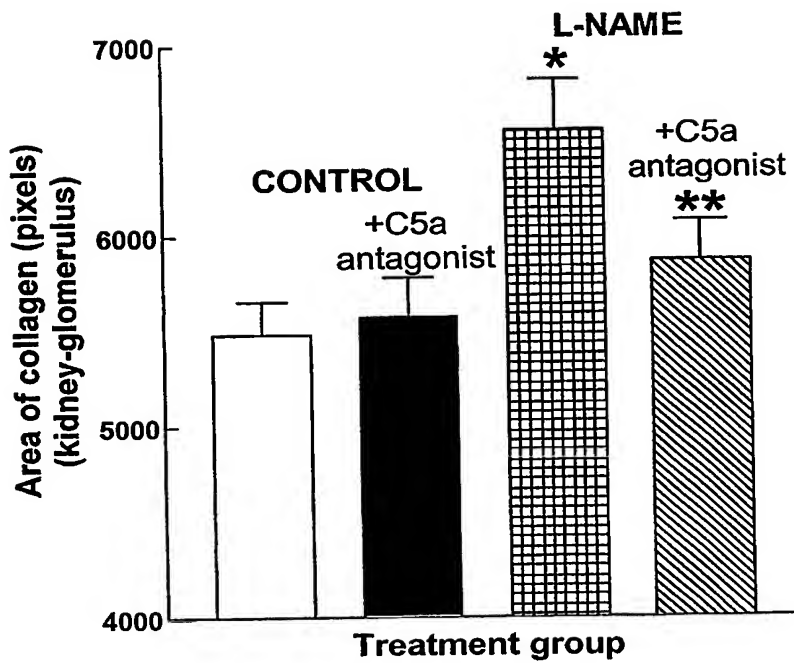


Figure 8a

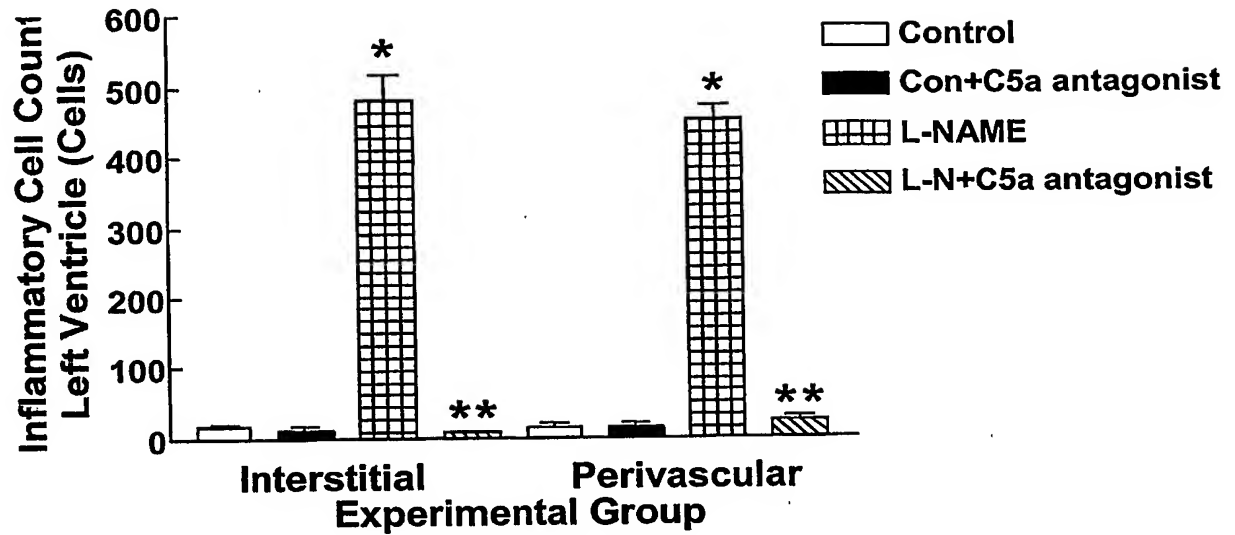


Figure 8b

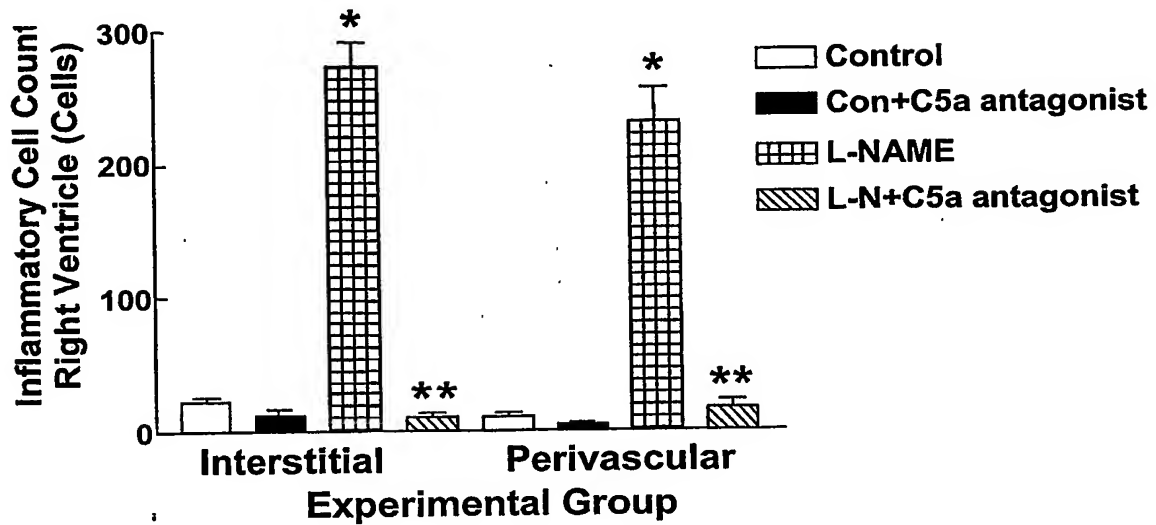
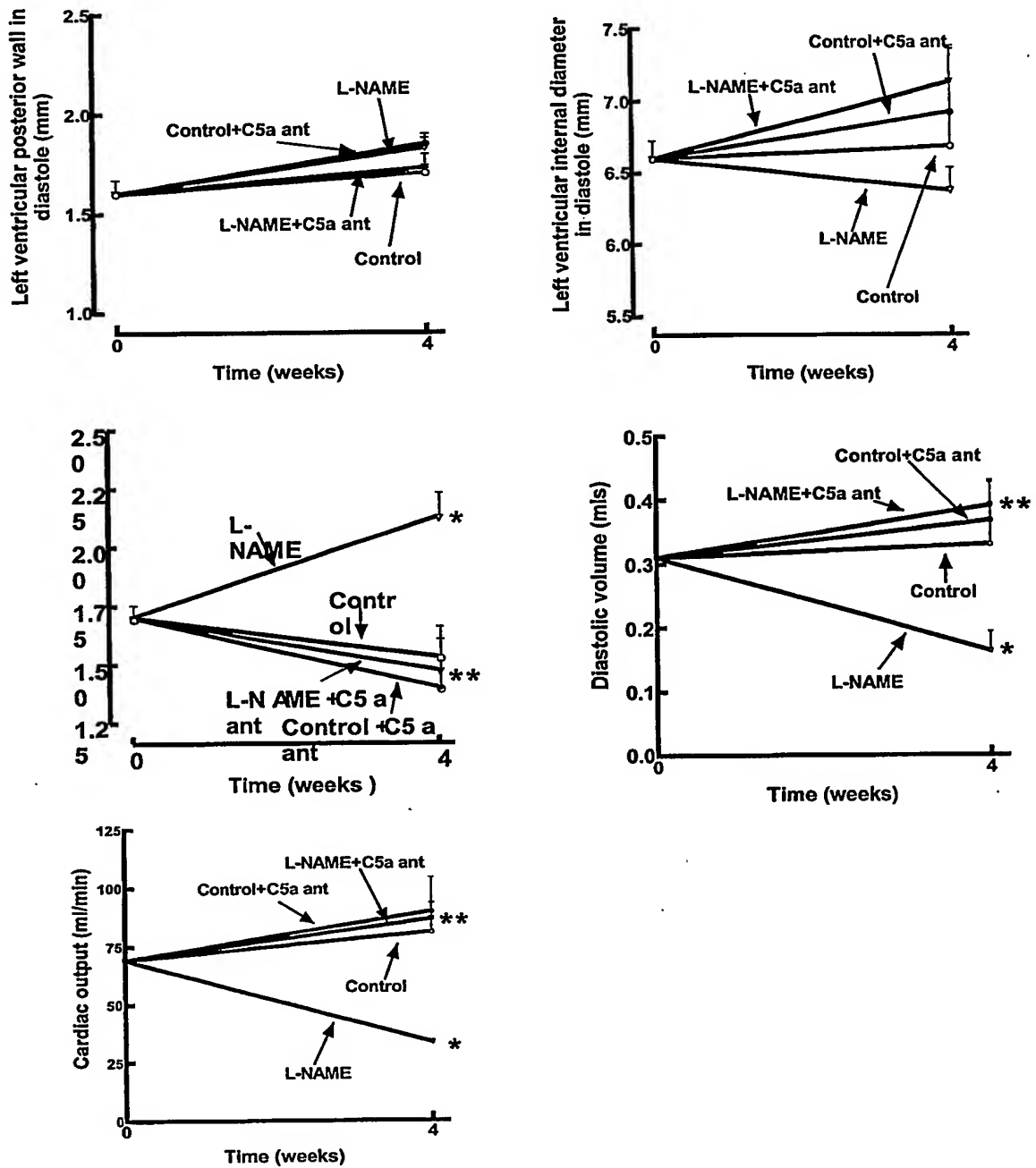


Figure 9



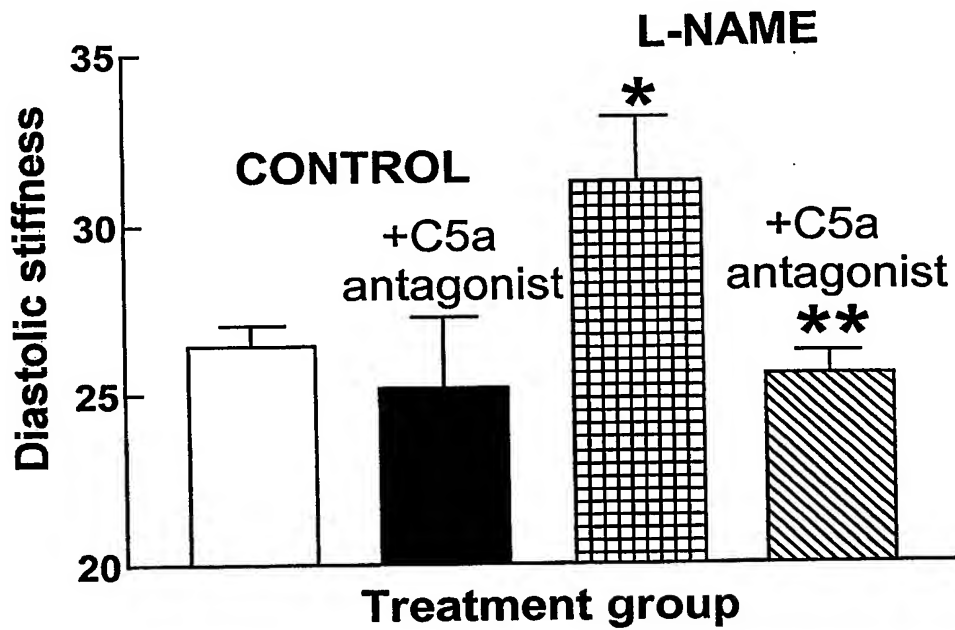


Figure 10

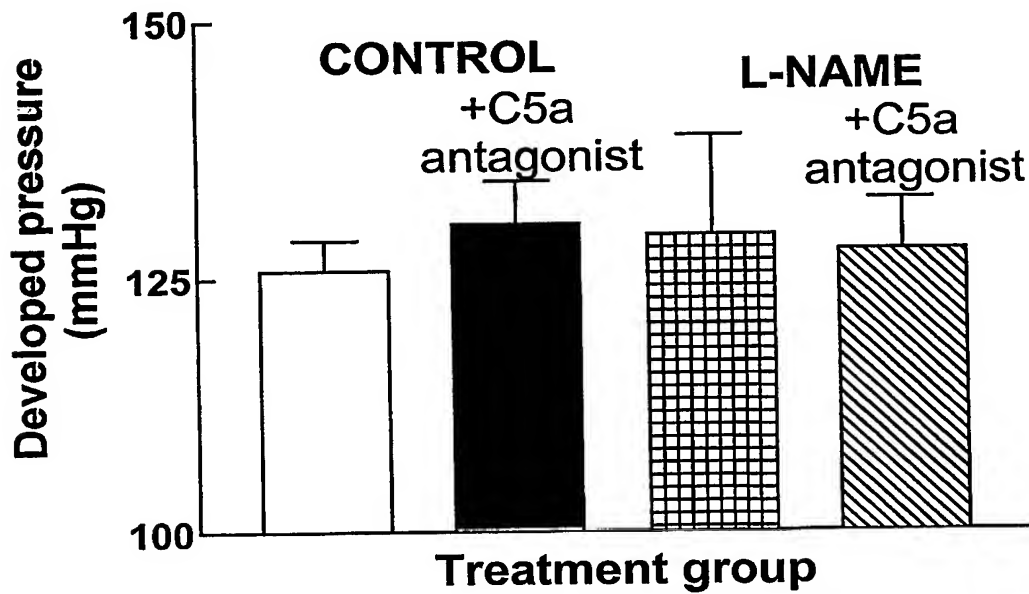


Figure 11